

## **REMARKS**

### ***Status of the Claims***

After amendment, claims 2-31 are pending in the present application. Claim 1 was previously cancelled. Claims 2, 4, 5 and 10-15 have been amended and new claims 16-31 have been added. No new matter has been added by way of the amendments or the new claims.

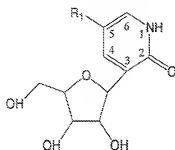
Claims 2, 4, 5 and 10-15 have been amended to further define and clarify the invention. Support for the amendment of claims 2, 4, 5 and 10-15 and for new claims 16-31 can at least be found in the specification at page 15, line 16 to page 16, line 1; page 17, lines 18-28; page 18, lines 1-10; Examples 11-14; Figures 3, 4, and 15-18; and original claims 2-10.

Based upon the above considerations, entry of the present Amendment is respectfully requested.

### ***Issue Under 35 U.S.C. § 103(a), Obviousness***

Claims 2-9 and 11-14 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Froehler *et al.*, U.S. Patent No. 6,447,998, U.S. Patent No. 6,495,672 or US Patent Publication No. 2003/0120065 (hereinafter collectively "Froehler"), in view of Ohtsuki *et al.*, "Unnatural Base Pairs for Specific Transcription," Proc. Natl. Acad. Sci., vol. 98, 2001, pp. 4922-25 (hereinafter "Ohtsuki") and Guo *et al.*, "Inhibition of DNA Polymerase Reactions by Pyrimidine Nucleotide Analogues Lacking the 2-Keto Group," Nucleic Acids Research, 1998, vol. 26, No. 8, pp. 1863-9 (hereinafter "Guo"). Applicants respectfully traverse.

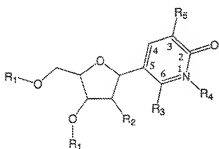
In the Office Action it is stated that Applicants' arguments and Dr. Hirao's Declaration of March 9, 2009 are based on the claimed invention being drawn to a compound having the following structure:



(Compound I)

The claimed invention is directed to a nucleoside or nucleotide having a “5-substituted-2-oxo(1H)-pyridin-3-yl” (emphasis added) group” as a base. One skilled in the art would recognize that the base is bound to the β-ribofranosyl sugar at the 3-position. The structure for Compound I, above, shows the numbered positions that correspond to those of the base of nucleosides and nucleotides of the claimed invention.

The compound taught by Froehler (Compound II, below) is distinct from the claimed invention in that the 3-position of the base is substituted, and the 5-position is bound to a sugar moiety.



(Compound II)

In contrast, nucleosides and nucleotides of the claimed invention have a base that is substituted at the 5-position, and a sugar (such as, ribose, phosphorylated ribose, deoxyribose, or phosphorylated deoxyribose) at the 3-position. This difference in structure between the

compounds taught by Froehler and the claimed invention is significant. Froehler, Ohtsuki, and Guo taken alone or together do not teach the claimed nucleosides and nucleotides having the recited substituents at the 5-position and a sugar (such as, ribose, phosphorylated ribose, deoxyribose, or phosphorylated deoxyribose) at the 3-position of the base.

Furthermore, although the nucleosides and nucleotides of the claimed invention have a keto group at the 2-position that causes them to appear to be similar in structure to natural bases like thymine and cytosine, the claimed nucleosides and nucleotides are incorporated only at positions complementary to 6-substituted 2-amino-purin-9-yl groups during replication and transcription. Substantially no incorporation of the claimed nucleosides and nucleotides is detected at positions complementary to natural bases after transcription, replication, or reverse transcription of a template is performed in the presence of the claimed nucleosides and nucleotides, which is a surprising result.

Furthermore, Froehler teaches triplex binding assays, and discloses oligomers, which can form triple helices by binding single-stranded DNA that contains the nucleoside taught by Froehler with specific DNA duplexes. In contrast, the nucleotides and nucleosides of the claimed invention form only duplexes.

In view of the discussion above, Applicants respectfully ask that the rejection of claims 2-9 and 11-14 under 35 U.S.C. § 103(a) as unpatentable over Froehler in view of Ohtsuki and Guo be withdrawn.

***Issue Under 35 U.S.C. § 112, First Paragraph, Written Description***

Claims 2-15 stand rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. The Examiner asserts that the claims contain subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. This is a new matter rejection. In the Office Action it is alleged that there is no support in the specification as filed for derivatives of dichloroacetyl group, fluorescein, 6-carboxyfluorescein, tetramethyl-6 carboxyrhodamine introduced at the 5-position of the nucleoside or nucleotide of the invention via a linker selected from an "aminoalkyl group, an aminoalkenyl group and an aminoalkynyl group."

After amendment, the claims no longer recite aminoalkynyl linkers linked to derivatives of dichloroacetyl group, fluorescein, 6-carboxyfluorescein, or tetramethyl-6 carboxyrhodamine. The use of aminoalkyl and aminoalkenyl linkers for modifying the base portion of a nucleoside or nucleotide is well-known in the art. Applicants submit herewith a copy of Goodman *et al.*, "*Structural requirements of olefinic 5-substituted deoxyuridines for antiherpes activity*," J. Med. Chem., vol. 26, 1983, pp. 1252-7, that discloses the use of such linkers in modifying uridine. Based on the specification and the state of the art at the time of the invention, one skilled in the art would have understood how to use such linkers to introduce a fluorescent molecule into a nucleoside or nucleotide, as described in the instant application.

In view of the discussion above, Applicants respectfully request withdrawal of the rejection of claims 2-15 under 35 U.S.C. § 112, first paragraph.

***Issue Under 35 U.S.C. § 112, Second Paragraph, Indefiniteness***

Claims 2-15 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention.

In the Office Action it is stated that the claims are indefinite because they include both the broad group of “biotin, dichloroacetyl, fluorescein, 6-carboxyfluorescein, tetramethyl-6-carboxyrhodamine, and derivatives thereof” and the narrower group of “biotin, dichloroacetyl, fluorescein, 6-carboxyfluorescein, tetramethyl-6-carboxyrhodamine, and derivatives thereof introduced via a linker selected from an aminoalkyl group, an aminoalkenyl group and an aminoalkynyl group.” Applicants have amended claims 2, 5 and 13-15 to distinguish the two groups, and make clear that they refer to two different types of substituents. Thus, Applicants respectfully request that the rejection of claims 2-15 under 35 U.S.C. § 112, second paragraph, be withdrawn.

**CONCLUSION**

In view of the above amendment, Applicants believe the pending application is in condition for allowance.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Stephanie Wardwell, Reg. No. 48,025, at the telephone number of the undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37.C.F.R. §§ 1.16 or 1.17; particularly, extension of time fees.

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Respectfully submitted,

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Attachment: Goodchild et al., J. Med. Chem. 26, pp. 1252-1257, 1983 (6 pages)

structure was refined further with anisotropic heavy atoms (C, N, and O) and isotropic hydrogen atoms to convergence at  $R = 0.043$ . At this point, analysis of the agreement between calculated and observed structure factors suggested the presence of secondary extinction ( $P_2 > F_0$ ), which affected the strongest low-order reflections: the (20-4), (209), and (202) reflections (with  $\sin \theta/\lambda \leq 0.131 \text{ \AA}^{-1}$ ). Correction for secondary extinction and a final cycle of refinement produced the residuals  $R = 0.036$  and weighted  $R = 0.047$  by using the 1952 observed reflections. The final difference map showed no significant peaks.

Computer programs used were from the CRYSTNET package.<sup>27</sup> The full-matrix least-squares program was UCLALS,<sup>28</sup> modified by H. L. Carroll.<sup>29</sup> Other programs for the structural solution and plotting (VIEW and BOOK) were developed at the Institute for Cancer Research.<sup>30</sup> The atomic scattering factors used for oxygen,

nitrogen, and carbon atoms<sup>31</sup> and for hydrogen atoms are listed in the literature.<sup>32</sup> Final positional and thermal parameters are listed in Table I. Lists of calculated and observed structure factors are available (see paragraph at end of paper regarding supplementary material).

**Acknowledgment.** This research was supported by American Cancer Society Grants IN-140 and BC-242, by National Institutes of Health Grants CA-10925, CA-22780, CA-06927, and RR 05539, and by an appropriation from the Commonwealth of Pennsylvania. I would especially like to thank Dr. Jenny Glusker for her valuable suggestions, patience, and support throughout this work. The author also acknowledges enlightening conversations with Drs. Murray-Rust and Liebman and the members of the crystallographic laboratories at The Institute for Cancer Research. In addition, special thanks are extended to the editor and reviewers for helpful comments and suggestions.

**Registry No.** Metyrapone, 54-36-4; cytochrome P-450, 9035-81-2.

**Supplementary Material Available:** Lists of calculated and observed structure factor amplitudes (3 pages). Ordering information is given on any current masthead page.

- (27) Bernstein, H. J.; Andrews, L. C.; Berman, H. M.; Bernstein, F. C.; Campbell, G. H.; Carrell, H. L.; Chasing, H. B.; Hamilton, W. C.; Jones, D. D.; Klunk, D.; Koetale, T. P.; Meyer, E. P.; Morimoto, C. N.; Sevan, S. S.; Stodola, R. K.; Strongson, M. M.; Silbaughby, T. V. *CRYSTNET: A Network of Intelligent Remote Graphics Terminals*; Brookhaven National Laboratory: Upton, NY, report BNL 18803, p 148.
- (28) Gantzel, P. K.; Sparks, R. A.; Long, R. E.; Trueblood, K. N. *UCLALS*, 1969; program in Fortran IV.
- (29) Carrell, H. L. *UCLALS: Modification of UCLALS*, Institute for Cancer Research: Philadelphia, 1975; program from the Institute for Cancer Research.
- (30) Carrell, H. L.; Shieh, H. S.; Takusagawa, F.; Wood, W. P., "Crystallographic Programs from the Institute for Cancer Research", Institute for Cancer Research: Philadelphia, 1978.

- (31) Cromer, D. T.; Waber, J. T. *Acta Crystallogr.* 1965, 18, 104.
- (32) Stewart, R. F.; Davidson, E. R.; Simpson, W. T. *J. Chem. Phys.* 1965, 42, 8175.

## Structural Requirements of Olefinic 5-Substituted Deoxyuridines for Antiherpetic Activity<sup>1</sup>

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A number of structurally related 5-substituted pyrimidine 2'-deoxyribonucleosides were synthesized and tested for antiviral activity against herpes simplex virus type 1 (HSV-1) in cell culture. A minimum inhibitory concentration was determined for each compound, and from a comparison of these values a number of conclusions were drawn with regard to those molecular features that enhance or reduce antiviral activity. Optimum inhibition of HSV-1 in cell culture occurred when the 5-substituent was unsaturated and conjugated with the pyrimidine ring, was not longer than four carbon atoms in length, had *E* stereochemistry, and included a hydrophobic, electronegative function but did not contain a branching point. Such features are contained in (E)-5-(2-bromovinyl)-2'-deoxyuridine, which was the most active of the compounds described.

Attempts to identify chemical antiviral agents that allow the effective and safe control of virus diseases of man have been largely unsuccessful. However, a number of nucleoside analogues are known that inhibit herpes simplex virus (HSV) replication.<sup>1,2</sup> The extent to which these inhibit virus growth without causing cellular toxicity reflects their degree of selectivity in reacting with virus-specific functions rather than interfering with cellular metabolism. There is evidence that the mechanism of action of these nucleoside analogues involves virus-coded enzymes important in DNA replication, and the exploitation of differences between virus-specific enzymes and the corresponding host cell enzymes provides a promising strategy in the search

for more effective and less toxic antiherpetic drugs.

The mechanism of action of the known antiviral nucleosides is, in many cases, not clearly defined. It has been proposed that some may interact with the virus-coded DNA polymerase<sup>3-5</sup> either as substrates or inhibitors, while others inhibit thymidylate synthetase.<sup>6</sup> However, such

<sup>1</sup> This work was first presented at the North American Medicinal Chemistry Symposium, June 1982.

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<sup>3</sup> Department of Biology.

(1) De Clercq, E.; Torrence, P. F. *J. Carbohydr. Nucleosides, Nucleotides* 1978, 5, 187-224.

(2) De Clercq, E. *Acta Microbiol. Acad. Sci. Hung.* 1981, 28, 289-308.

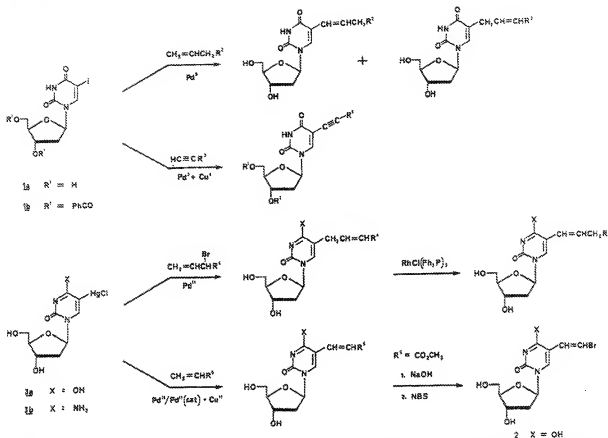
(3) Furman, P. A.; St. Clair, M. H.; Fyfe, J. A.; Ridgout, J. L.; Keller, P. M.; Ellison, G. B. *J. Virol.* 1979, 32, 72-77.

(4) Aliaudeen, H. S.; Kozarich, J. W.; Bertino, J. R.; De Clercq, E. *Proc. Natl. Acad. Sci. U.S.A.* 1981, 78, 2698-2702.

(5) Ruth, J. L.; Cheng, Y.-C. *J. Biol. Chem.* 1982, 257, 10261-10265.

(6) De Clercq, E. *Methods Find. Exp. Clin. Pharmacol.* 1980, 2, 253-267.

Scheme I



interactions require that the compounds first be phosphorylated. This is best accomplished intracellularly, since nucleosides are inefficiently taken up by cells and, therefore, have little potential for therapeutic use.<sup>7,8</sup> Certain herpes viruses, such as HSV and Varicella-Zoster virus (VZV), code for a unique thymidine kinase<sup>9,10</sup> that increases the ability of infected cells to phosphorylate thymidine compared with uninfected cells. Consequently, analogues of thymidine are potentially more active as antimetabolites in such virus-infected cells than in uninfected cells and those compounds that interact specifically with herpes virus induced, but not host cell, thymidine kinase would be expected to exhibit a high degree of selectivity of drug action.

The thymidine kinase of HSV type 1 (HSV-1) is particularly tolerant of pyrimidines substituted at the 5-position of the base, and many such compounds show antitherpes activity. 5-Iodo-2'-deoxyuridine (IDU, 1a) inhibits HSV-1 replication and is used in certain clinical situations, but it has the disadvantage of being phosphorylated in uninfected cells, is toxic, and may be mutagenic and teratogenic (for review, see ref 11). (E)-5-(2-Bromovinyl)-2'-deoxyuridine (BVDU, 2) is a better inhibitor of HSV-1 than is IDU,<sup>12,13</sup> and, with a high affinity for HSV

thymidine kinase and a low affinity for the corresponding cell enzyme,<sup>14</sup> it is more selective in its action. Many of the substituents introduced at the 5-position of deoxyuridine that result in antitherpes activity are either unsaturated or electronegative or both. This paper reports the antitherpes activity of a series of pyrimidine 2'-deoxynucleosides with unsaturated substituents at the 5-position (Table I). Certain features in these substituents are identified as promoting antiviral activity.

**Chemistry.** One of the most useful methods for the introduction of unsaturated substituents into the 5-position of pyrimidines is by a modification of the Heck reaction.<sup>15</sup> This involves the palladium-catalyzed addition of alkenes or alkynes to either 5-halogenated (e.g., 1) or 5-mercuri (e.g., 3) derivatives, the former generally requiring harsher reaction conditions. In agreement with the results of Bergstrom et al.,<sup>16</sup> we have found this reaction to be most effective with alkenes having an electron-withdrawing substituent or a leaving group in an allylic position. Although the latter case produces 2-alkenyl substituents (Scheme I), isomerization to the conjugated product is

(7) Plagemann, P. G. W.; Wohlhueter, R. M. *Curr. Top. Membr. Transp.* 1980, 14, 225-330.

(8) Montgomery, J. A. *Prog. Med. Chem.* 1970, 7, 69-123.

(9) Klemperer, H. G.; Haynes, G. R.; Shelden, W. I. H.; Watson, D. H. *Virology* 1967, 31, 120-128.

(10) Cheng, Y.-C.; Tsou, T. Y.; Hackstadt, T.; Mallavia, L. P. *J. Virol.* 1979, 31, 172-177.

(11) Gox, B. *Pharmacol. Rev.* 1978, 29, 249-272.

(12) De Clercq, E.; Descamps, J.; De Somer, P.; Barr, P. J.; Jones, A. S.; Walker, R. T. *Proc. Natl. Acad. Sci. U.S.A.* 1978, 76, 2947-2951.

(13) De Clercq, E.; Descamps, J.; Verhelst, G.; Walker, R. T.; Jones, A. S.; Torrence, P. F.; Shugar, D. J. *Infect. Dis.* 1980, 141, 565-574.

(14) Cheng, Y.-C.; Dutschman, G.; De Clercq, E.; Jones, A. S.; Rahim, S. G.; Verhelst, G.; Walker, R. T. *Mol. Pharmacol.* 1981, 20, 230-233.

(15) Heck, R. F. *Acc. Chem. Res.* 1979, 12, 146-151.

(16) Bergstrom, D. E.; Ruth, J. L.; Warwick, P. J. *Org. Chem.* 1961, 46, 1432-1441.

Table 1. Physical Characteristics and Antiviral Activity of 5-Substituted Deoxypyrimidine Nucleosides

no.	X	R	mp, °C	nuclyotin solvent	UV $\lambda_{max}$ , nm	NMR $\delta^a$ , Hz	formula	anal.	MIC, $\mu$ g/mL
2	OH	(E)-CH=CHBr <sup>b</sup>	141-143	H <sub>2</sub> O	296	17	C <sub>8</sub> H <sub>8</sub> BrN <sub>2</sub> O	C, H, N, Br	0.005
3	OH	(Z)-CH=CHBr <sup>c</sup>	184.5-185	CH <sub>3</sub> CN	230, 291 <sup>d</sup>	8	C <sub>8</sub> H <sub>8</sub> BrN <sub>2</sub> O	C, H, N, Br	0.1
4	OH	(E)-CH=CHCH <sub>3</sub>	215-220	MeOH	296	16	C <sub>8</sub> H <sub>9</sub> BrN <sub>2</sub> O	C, H, N, Br	0.5
5	OH	(E)-CH=CHCH <sub>3</sub>	>310	H <sub>2</sub> O	271, 322	18	C <sub>8</sub> H <sub>9</sub> N <sub>2</sub> O <sub>2</sub> S	C, H, N, S	>25
6	OH	(E)-CH=CHCO <sub>2</sub> CH <sub>3</sub>	168-170	H <sub>2</sub> O	304 (40), 301	16	C <sub>10</sub> H <sub>11</sub> N <sub>2</sub> O <sub>3</sub>	C, H, N	10
7	OH	(E)-CH=CHCO <sub>2</sub> CH <sub>3</sub>	124.5-126	CH <sub>3</sub> CN	269	16	C <sub>10</sub> H <sub>11</sub> N <sub>2</sub> O <sub>3</sub>	C, H, N	100
8	OH	(E)-CH=CHCH <sub>3</sub>	191-192	CH <sub>3</sub> CN	292	16	C <sub>8</sub> H <sub>9</sub> N <sub>2</sub> O	C, H, N	100.5
9	OH	(E)-CH=CHCH <sub>3</sub>	171.5-173.5	H <sub>2</sub> O/MeOH	286	11	C <sub>8</sub> H <sub>9</sub> N <sub>2</sub> O	C, H, N	100
10	OH	(E)-CH=CHCH <sub>3</sub>	161-162	H <sub>2</sub> O	228, 293	11	C <sub>8</sub> H <sub>9</sub> N <sub>2</sub> O	C, H, N	1
11	OH	(E)-CH=CHCH <sub>3</sub>	117-118	CH <sub>3</sub> CN/H <sub>2</sub> O	295, 351	16	C <sub>8</sub> H <sub>9</sub> N <sub>2</sub> O <sub>2</sub> S	C, H, N	100
12	OH	(E)-CH=CHCH <sub>3</sub>	197-201	CH <sub>3</sub> CN/MeOH	296	16	C <sub>8</sub> H <sub>9</sub> N <sub>2</sub> O	C, H, N	80
13	SH	(E)-CH=CHCH <sub>3</sub>	161.5-162.5	CH <sub>3</sub> CN/MeOH	290	11	C <sub>8</sub> H <sub>9</sub> N <sub>2</sub> O	C, H, N	>100
14	NH <sub>2</sub>	(Z)-CH=CHCH <sub>3</sub>	79-81	CH <sub>3</sub> CN	270	17	C <sub>8</sub> H <sub>9</sub> N <sub>3</sub> O	C, H, N	0.1
15	NH <sub>2</sub>	(Z)-CH=CHCH <sub>3</sub>	130-131	H <sub>2</sub> O	235, 285	18	C <sub>8</sub> H <sub>9</sub> BrN <sub>2</sub> O	C, H, N, Br	1
16	OH	(Z)-CH=CHCH <sub>3</sub>	194-196	H <sub>2</sub> O	233, 293	16	C <sub>8</sub> H <sub>9</sub> BrN <sub>2</sub> O	C, H, N, Br	>100
17	OH	(Z)-CH=CHCH <sub>3</sub>	178-179	H <sub>2</sub> O	234, 298	16	C <sub>8</sub> H <sub>9</sub> BrN <sub>2</sub> O	C, H, N, Br	>100
18	OH	(E)-CH=CHCH <sub>3</sub>	181-182	H <sub>2</sub> O	263, 302	296	C <sub>8</sub> H <sub>9</sub> N <sub>2</sub> O	C, H, N	>100
19	OH	(E)-CH=CHCH <sub>3</sub>	148-150	CH <sub>3</sub> CN	270	ND <sup>p</sup>	C <sub>8</sub> H <sub>9</sub> N <sub>2</sub> O	C, H, N	>100
20	OH	(E)-CH=CHCH <sub>3</sub>	189-190.5	H <sub>2</sub> O	295	16	C <sub>8</sub> H <sub>9</sub> N <sub>2</sub> O	C, H, N	0.5
21	OH	(E)-CH=CHCH <sub>3</sub>	121	CH <sub>3</sub> CN	270	16	C <sub>8</sub> H <sub>9</sub> N <sub>2</sub> O	C, H, N	10
22	OH	(E)-CH=CHCH <sub>3</sub>	208-209	H <sub>2</sub> O	230, 286	16	C <sub>8</sub> H <sub>9</sub> N <sub>2</sub> O	C, H, N	>50
23	OH	(E)-CH=CHCH <sub>3</sub>	152-155	H <sub>2</sub> O	279, 290	16	C <sub>8</sub> H <sub>9</sub> N <sub>2</sub> O	C, H, N	60
24	OH	(E)-CH=CHCH <sub>3</sub>	129-130	H <sub>2</sub> O	241, 296	16	C <sub>8</sub> H <sub>9</sub> N <sub>2</sub> O	C, H, N	>100
25	OH	(E)-CH=CHCH <sub>3</sub>	136-140	H <sub>2</sub> O/MeOH	241, 297	16	C <sub>8</sub> H <sub>9</sub> N <sub>2</sub> O	C, H, N	>100
26	OH	(E)-CH=CHCH <sub>3</sub>	150-152	H <sub>2</sub> O	239, 294	16	C <sub>8</sub> H <sub>9</sub> N <sub>2</sub> O	C, H, N	>100
27	OH	(E)-CH=CHCH <sub>3</sub>	186	MeOH	340	15, 15	C <sub>8</sub> H <sub>9</sub> N <sub>2</sub> O	C, H, N	>100

<sup>a</sup> Coupling constants for vinyl protons in R. <sup>b</sup> See ref 18. <sup>c</sup> This material was a gift from Prof. A. S. Jones; see ref 19. <sup>d</sup> Values reported in the literature<sup>19</sup> are 234, 295 nm for <sup>1</sup>H<sub>2</sub>O. <sup>e</sup> See ref 16. <sup>f</sup> See ref 16. <sup>g</sup> See ref 16. <sup>h</sup> See ref 16. <sup>i</sup> See ref 16. <sup>j</sup> See ref 16. <sup>k</sup> See ref 16. <sup>l</sup> See ref 16. <sup>m</sup> See ref 16. <sup>n</sup> See ref 16. <sup>o</sup> See ref 16. <sup>p</sup> See ref 16. <sup>q</sup> See ref 16. <sup>r</sup> See ref 16. <sup>s</sup> See ref 16. <sup>t</sup> See ref 16. <sup>u</sup> See ref 16. <sup>v</sup> See ref 16. <sup>w</sup> See ref 16. <sup>x</sup> See ref 16. <sup>y</sup> See ref 16. <sup>z</sup> See ref 16. <sup>aa</sup> See ref 16. <sup>ab</sup> See ref 16. <sup>ac</sup> See ref 16. <sup>ad</sup> See ref 16. <sup>ae</sup> See ref 16. <sup>af</sup> See ref 16. <sup>ag</sup> See ref 16. <sup>ah</sup> See ref 16. <sup>ai</sup> See ref 16. <sup>aj</sup> See ref 16. <sup>ak</sup> See ref 16. <sup>al</sup> See ref 16. <sup>am</sup> See ref 16. <sup>an</sup> See ref 16. <sup>ao</sup> See ref 16. <sup>ap</sup> See ref 16. <sup>aq</sup> See ref 16. <sup>ar</sup> See ref 16. <sup>as</sup> See ref 16. <sup>at</sup> See ref 16. <sup>au</sup> See ref 16. <sup>av</sup> See ref 16. <sup>aw</sup> See ref 16. <sup>ax</sup> See ref 16. <sup>ay</sup> See ref 16. <sup>az</sup> See ref 16. <sup>ba</sup> See ref 16. <sup>bb</sup> See ref 16. <sup>bc</sup> See ref 16. <sup>bd</sup> See ref 16. <sup>be</sup> See ref 16. <sup>bf</sup> See ref 16. <sup>bg</sup> See ref 16. <sup>bh</sup> See ref 16. <sup>bi</sup> See ref 16. <sup>bj</sup> See ref 16. <sup>bk</sup> See ref 16. <sup>bl</sup> See ref 16. <sup>bm</sup> See ref 16. <sup>bn</sup> See ref 16. <sup>bo</sup> See ref 16. <sup>bp</sup> See ref 16. <sup>bq</sup> See ref 16. <sup>br</sup> See ref 16. <sup>bs</sup> See ref 16. <sup>bt</sup> See ref 16. <sup>bu</sup> See ref 16. <sup>bv</sup> See ref 16. <sup>bw</sup> See ref 16. <sup>bx</sup> See ref 16. <sup>by</sup> See ref 16. <sup>bz</sup> See ref 16. <sup>ca</sup> See ref 16. <sup>cb</sup> See ref 16. <sup>cc</sup> See ref 16. <sup>cd</sup> See ref 16. <sup>ce</sup> See ref 16. <sup>cf</sup> See ref 16. <sup>cg</sup> See ref 16. <sup>ch</sup> See ref 16. <sup>ci</sup> See ref 16. <sup>cj</sup> See ref 16. <sup>ck</sup> See ref 16. <sup>cl</sup> See ref 16. <sup>cm</sup> See ref 16. <sup>cn</sup> See ref 16. <sup>co</sup> See ref 16. <sup>cp</sup> See ref 16. <sup>cq</sup> See ref 16. <sup>cr</sup> See ref 16. <sup>cs</sup> See ref 16. <sup>ct</sup> See ref 16. <sup>cu</sup> See ref 16. <sup>cv</sup> See ref 16. <sup>cw</sup> See ref 16. <sup>cx</sup> See ref 16. <sup>cy</sup> See ref 16. <sup>cz</sup> See ref 16. <sup>da</sup> See ref 16. <sup>db</sup> See ref 16. <sup>dc</sup> See ref 16. <sup>dd</sup> See ref 16. <sup>de</sup> See ref 16. <sup>df</sup> See ref 16. <sup>dg</sup> See ref 16. <sup>dh</sup> See ref 16. <sup>di</sup> See ref 16. <sup>dj</sup> See ref 16. <sup>dk</sup> See ref 16. <sup>dl</sup> See ref 16. <sup>dm</sup> See ref 16. <sup>dn</sup> See ref 16. <sup>do</sup> See ref 16. <sup>dp</sup> See ref 16. <sup>dq</sup> See ref 16. <sup>dr</sup> See ref 16. <sup>ds</sup> See ref 16. <sup>dt</sup> See ref 16. <sup>du</sup> See ref 16. <sup>dv</sup> See ref 16. <sup>dw</sup> See ref 16. <sup>dx</sup> See ref 16. <sup>dy</sup> See ref 16. <sup>dz</sup> See ref 16. <sup>ea</sup> See ref 16. <sup>eb</sup> See ref 16. <sup>ec</sup> See ref 16. <sup>ed</sup> See ref 16. <sup>ee</sup> See ref 16. <sup>ef</sup> See ref 16. <sup>eg</sup> See ref 16. <sup>eh</sup> See ref 16. <sup>ei</sup> See ref 16. <sup>ej</sup> See ref 16. <sup>ek</sup> See ref 16. <sup>el</sup> See ref 16. <sup>em</sup> See ref 16. <sup>en</sup> See ref 16. <sup>eo</sup> See ref 16. <sup>ep</sup> See ref 16. <sup>eq</sup> See ref 16. <sup>er</sup> See ref 16. <sup>es</sup> See ref 16. <sup>et</sup> See ref 16. <sup>eu</sup> See ref 16. <sup>ev</sup> See ref 16. <sup>ew</sup> See ref 16. <sup>ex</sup> See ref 16. <sup>ey</sup> See ref 16. <sup>ez</sup> See ref 16. <sup>fa</sup> See ref 16. <sup>fb</sup> See ref 16. <sup>fc</sup> See ref 16. <sup>fd</sup> See ref 16. <sup>fe</sup> See ref 16. <sup>ff</sup> See ref 16. <sup>fg</sup> See ref 16. <sup>fh</sup> See ref 16. <sup>fi</sup> See ref 16. <sup>fj</sup> See ref 16. <sup>fk</sup> See ref 16. <sup>fl</sup> See ref 16. <sup>fm</sup> See ref 16. <sup>fn</sup> See ref 16. <sup>fo</sup> See ref 16. <sup>fp</sup> See ref 16. <sup>fq</sup> See ref 16. <sup>fr</sup> See ref 16. <sup>fs</sup> See ref 16. <sup>ft</sup> See ref 16. <sup>fu</sup> See ref 16. <sup>fv</sup> See ref 16. <sup>fw</sup> See ref 16. <sup>fx</sup> See ref 16. <sup>fy</sup> See ref 16. <sup>fz</sup> See ref 16. <sup>ga</sup> See ref 16. <sup>gb</sup> See ref 16. <sup>gc</sup> See ref 16. <sup>gd</sup> See ref 16. <sup>ge</sup> See ref 16. <sup>gf</sup> See ref 16. <sup>gg</sup> See ref 16. <sup>gh</sup> See ref 16. <sup>gi</sup> See ref 16. <sup>gj</sup> See ref 16. <sup>gk</sup> See ref 16. <sup>gl</sup> See ref 16. <sup>gm</sup> See ref 16. <sup>gn</sup> See ref 16. <sup>go</sup> See ref 16. <sup>gp</sup> See ref 16. <sup>gq</sup> See ref 16. <sup>gr</sup> See ref 16. <sup>gs</sup> See ref 16. <sup>gt</sup> See ref 16. <sup>gu</sup> See ref 16. <sup>gv</sup> See ref 16. <sup>gw</sup> See ref 16. <sup>gx</sup> See ref 16. <sup>gy</sup> See ref 16. <sup>gz</sup> See ref 16. <sup>ha</sup> See ref 16. <sup>hb</sup> See ref 16. <sup>hc</sup> See ref 16. <sup>hd</sup> See ref 16. <sup>he</sup> See ref 16. <sup>hf</sup> See ref 16. <sup>hg</sup> See ref 16. <sup>hh</sup> See ref 16. <sup>hi</sup> See ref 16. <sup>hj</sup> See ref 16. <sup>hk</sup> See ref 16. <sup>hl</sup> See ref 16. <sup>hm</sup> See ref 16. <sup>hn</sup> See ref 16. <sup>ho</sup> See ref 16. <sup>hp</sup> See ref 16. <sup>hq</sup> See ref 16. <sup>hr</sup> See ref 16. <sup>hs</sup> See ref 16. <sup>ht</sup> See ref 16. <sup>hu</sup> See ref 16. <sup>hv</sup> See ref 16. <sup>hw</sup> See ref 16. <sup>hx</sup> See ref 16. <sup>hy</sup> See ref 16. <sup>hz</sup> See ref 16. <sup>ia</sup> See ref 16. <sup>ib</sup> See ref 16. <sup>ic</sup> See ref 16. <sup>id</sup> See ref 16. <sup>ie</sup> See ref 16. <sup>if</sup> See ref 16. <sup>ig</sup> See ref 16. <sup>ih</sup> See ref 16. <sup>ii</sup> See ref 16. <sup>ij</sup> See ref 16. <sup>ik</sup> See ref 16. <sup>il</sup> See ref 16. <sup>im</sup> See ref 16. <sup>in</sup> See ref 16. <sup>io</sup> See ref 16. <sup>ip</sup> See ref 16. <sup>iq</sup> See ref 16. <sup>ir</sup> See ref 16. <sup>is</sup> See ref 16. <sup>it</sup> See ref 16. <sup>iu</sup> See ref 16. <sup>iv</sup> See ref 16. <sup>iw</sup> See ref 16. <sup>ix</sup> See ref 16. <sup>iy</sup> See ref 16. <sup>iz</sup> See ref 16. <sup>ja</sup> See ref 16. <sup>jb</sup> See ref 16. <sup>jc</sup> See ref 16. <sup>jd</sup> See ref 16. <sup>je</sup> See ref 16. <sup>jf</sup> See ref 16. <sup>jj</sup> See ref 16. <sup>jk</sup> See ref 16. <sup>jl</sup> See ref 16. <sup>jm</sup> See ref 16. <sup>jn</sup> See ref 16. <sup>jo</sup> See ref 16. <sup>jp</sup> See ref 16. <sup>jq</sup> See ref 16. <sup>jr</sup> See ref 16. <sup>js</sup> See ref 16. <sup>jt</sup> See ref 16. <sup>ju</sup> See ref 16. <sup>jv</sup> See ref 16. <sup>jw</sup> See ref 16. <sup>jx</sup> See ref 16. <sup>jy</sup> See ref 16. <sup>jz</sup> See ref 16. <sup>ka</sup> See ref 16. <sup>kb</sup> See ref 16. <sup>kc</sup> See ref 16. <sup>kd</sup> See ref 16. <sup>ke</sup> See ref 16. <sup>kf</sup> See ref 16. <sup>kg</sup> See ref 16. <sup>kh</sup> See ref 16. <sup>ki</sup> See ref 16. <sup>kj</sup> See ref 16. <sup>kl</sup> See ref 16. <sup>km</sup> See ref 16. <sup>kn</sup> See ref 16. <sup>ko</sup> See ref 16. <sup>kp</sup> See ref 16. <sup>kq</sup> See ref 16. <sup>kr</sup> See ref 16. <sup>ks</sup> See ref 16. <sup>kt</sup> See ref 16. <sup>ku</sup> See ref 16. <sup>kv</sup> See ref 16. <sup>kx</sup> See ref 16. <sup>ky</sup> See ref 16. <sup>kz</sup> See ref 16. <sup>la</sup> See ref 16. <sup>lb</sup> See ref 16. <sup>lc</sup> See ref 16. <sup>ld</sup> See ref 16. <sup>le</sup> See ref 16. <sup>lf</sup> See ref 16. <sup>lg</sup> See ref 16. <sup>lh</sup> See ref 16. <sup>li</sup> See ref 16. <sup>lj</sup> See ref 16. <sup>lk</sup> See ref 16. <sup>lm</sup> See ref 16. <sup>ln</sup> See ref 16. <sup>lo</sup> See ref 16. <sup>lp</sup> See ref 16. <sup>lq</sup> See ref 16. <sup>lr</sup> See ref 16. <sup>ls</sup> See ref 16. <sup>lt</sup> See ref 16. <sup>lu</sup> See ref 16. <sup>lv</sup> See ref 16. <sup>lw</sup> See ref 16. <sup>lx</sup> See ref 16. <sup>ly</sup> See ref 16. <sup>lz</sup> See ref 16. <sup>ma</sup> See ref 16. <sup>mb</sup> See ref 16. <sup>mc</sup> See ref 16. <sup>md</sup> See ref 16. <sup>me</sup> See ref 16. <sup>mf</sup> See ref 16. <sup>mg</sup> See ref 16. <sup>mh</sup> See ref 16. <sup>mi</sup> See ref 16. <sup>mj</sup> See ref 16. <sup>mk</sup> See ref 16. <sup>ml</sup> See ref 16. <sup>mm</sup> See ref 16. <sup>mn</sup> See ref 16. <sup>mo</sup> See ref 16. <sup>mp</sup> See ref 16. <sup>mq</sup> See ref 16. <sup>mr</sup> See ref 16. <sup>ms</sup> See ref 16. <sup>mt</sup> See ref 16. <sup>mu</sup> See ref 16. <sup>mv</sup> See ref 16. <sup>mw</sup> See ref 16. <sup>mx</sup> See ref 16. <sup>my</sup> See ref 16. <sup>mz</sup> See ref 16. <sup>na</sup> See ref 16. <sup>nb</sup> See ref 16. <sup>nc</sup> See ref 16. <sup>nd</sup> See ref 16. <sup>ne</sup> See ref 16. <sup>nf</sup> See ref 16. <sup>ng</sup> See ref 16. <sup>nh</sup> See ref 16. <sup>ni</sup> See ref 16. <sup>nj</sup> See ref 16. <sup>nk</sup> See ref 16. <sup>nl</sup> See ref 16. <sup>nm</sup> See ref 16. <sup>no</sup> See ref 16. <sup>np</sup> See ref 16. <sup>nq</sup> See ref 16. <sup>nr</sup> See ref 16. <sup>ns</sup> See ref 16. <sup>nt</sup> See ref 16. <sup>nu</sup> See ref 16. <sup>nv</sup> See ref 16. <sup>nw</sup> See ref 16. <sup>nx</sup> See ref 16. <sup>ny</sup> See ref 16. <sup>nz</sup> See ref 16. <sup>oa</sup> See ref 16. <sup>ob</sup> See ref 16. <sup>oc</sup> See ref 16. <sup>od</sup> See ref 16. <sup>oe</sup> See ref 16. <sup>of</sup> See ref 16. <sup>og</sup> See ref 16. <sup>oh</sup> See ref 16. <sup>oi</sup> See ref 16. <sup>oj</sup> See ref 16. <sup>ok</sup> See ref 16. <sup>ol</sup> See ref 16. <sup>om</sup> See ref 16. <sup>on</sup> See ref 16. <sup>oo</sup> See ref 16. <sup>op</sup> See ref 16. <sup>oq</sup> See ref 16. <sup>or</sup> See ref 16. <sup>os</sup> See ref 16. <sup>ot</sup> See ref 16. <sup>ou</sup> See ref 16. <sup>ov</sup> See ref 16. <sup>ow</sup> See ref 16. <sup>ox</sup> See ref 16. <sup>oy</sup> See ref 16. <sup>oz</sup> See ref 16. <sup>pa</sup> See ref 16. <sup>pb</sup> See ref 16. <sup>pc</sup> See ref 16. <sup>pd</sup> See ref 16. <sup>pe</sup> See ref 16. <sup>pf</sup> See ref 16. <sup>pg</sup> See ref 16. <sup>ph</sup> See ref 16. <sup>pi</sup> See ref 16. <sup>pj</sup> See ref 16. <sup>pk</sup> See ref 16. <sup>pl</sup> See ref 16. <sup>pm</sup> See ref 16. <sup>pn</sup> See ref 16. <sup>po</sup> See ref 16. <sup>pp</sup> See ref 16. <sup>pq</sup> See ref 16. <sup>pr</sup> See ref 16. <sup>ps</sup> See ref 16. <sup>pt</sup> See ref 16. <sup>pu</sup> See ref 16. <sup>pv</sup> See ref 16. <sup>pw</sup> See ref 16. <sup>px</sup> See ref 16. <sup>py</sup> See ref 16. <sup>pz</sup> See ref 16. <sup>qa</sup> See ref 16. <sup>qb</sup> See ref 16. <sup>qc</sup> See ref 16. <sup>qd</sup> See ref 16. <sup>qe</sup> See ref 16. <sup>qf</sup> See ref 16. <sup>qg</sup> See ref 16. <sup>qh</sup> See ref 16. <sup>qi</sup> See ref 16. <sup>qj</sup> See ref 16. <sup>ql</sup> See ref 16. <sup>qm</sup> See ref 16. <sup>qn</sup> See ref 16. <sup>qo</sup> See ref 16. <sup>qp</sup> See ref 16. <sup>qq</sup> See ref 16. <sup>qr</sup> See ref 16. <sup>qs</sup> See ref 16. <sup>qt</sup> See ref 16. <sup>qu</sup> See ref 16. <sup>qv</sup> See ref 16. <sup>qw</sup> See ref 16. <sup>qx</sup> See ref 16. <sup>qy</sup> See ref 16. <sup>qz</sup> See ref 16. <sup>ra</sup> See ref 16. <sup>rb</sup> See ref 16. <sup>rc</sup> See ref 16. <sup>rd</sup> See ref 16. <sup>re</sup> See ref 16. <sup>rf</sup> See ref 16. <sup>rg</sup> See ref 16. <sup>rh</sup> See ref 16. <sup>ri</sup> See ref 16. <sup>rj</sup> See ref 16. <sup>rk</sup> See ref 16. <sup>rl</sup> See ref 16. <sup>rm</sup> See ref 16. <sup>rn</sup> See ref 16. <sup>ro</sup> See ref 16. <sup>rp</sup> See ref 16. <sup>rq</sup> See ref 16. <sup>rr</sup> See ref 16. <sup>rs</sup> See ref 16. <sup>rt</sup> See ref 16. <sup>ru</sup> See ref 16. <sup>rv</sup> See ref 16. <sup>rw</sup> See ref 16. <sup>rx</sup> See ref 16. <sup>ry</sup> See ref 16. <sup>rz</sup> See ref 16. <sup>sa</sup> See ref 16. <sup>sb</sup> See ref 16. <sup>sc</sup> See ref 16. <sup>sd</sup> See ref 16. <sup>se</sup> See ref 16. <sup>sf</sup> See ref 16. <sup>sg</sup> See ref 16. <sup>sh</sup> See ref 16. <sup>si</sup> See ref 16. <sup>sj</sup> See ref 16. <sup>sk</sup> See ref 16. <sup>sl</sup> See ref 16. <sup>sm</sup> See ref 16. <sup>sn</sup> See ref 16. <sup>so</sup> See ref 16. <sup>sp</sup> See ref 16. <sup>sq</sup> See ref 16. <sup>sr</sup> See ref 16. <sup>ss</sup> See ref 16. <sup>st</sup> See ref 16. <sup>su</sup> See ref 16. <sup>sv</sup> See ref 16. <sup>sw</sup> See ref 16. <sup>sx</sup> See ref 16. <sup>sy</sup> See ref 16. <sup>sz</sup> See ref 16. <sup>ta</sup> See ref 16. <sup>tb</sup> See ref 16. <sup>tc</sup> See ref 16. <sup>td</sup> See ref 16. <sup>te</sup> See ref 16. <sup>tf</sup> See ref 16. <sup>tg</sup> See ref 16. <sup>th</sup> See ref 16. <sup>ti</sup> See ref 16. <sup>tj</sup> See ref 16. <sup>tk</sup> See ref 16. <sup>tl</sup> See ref 16. <sup>tm</sup> See ref 16. <sup>tn</sup> See ref 16. <sup>to</sup> See ref 16. <sup>tp</sup> See ref 16. <sup>tr</sup> See ref 16. <sup>ts</sup> See ref 16. <sup>tu</sup> See ref 16. <sup>tv</sup> See ref 16. <sup>tw</sup> See ref 16. <sup>tx</sup> See ref 16. <sup>ty</sup> See ref 16. <sup>tz</sup> See ref 16. <sup>ua</sup> See ref 16. <sup>ub</sup> See ref 16. <sup>uc</sup> See ref 16. <sup>ud</sup> See ref 16. <sup>ue</sup> See ref 16. <sup>uf</sup> See ref 16. <sup>ug</sup> See ref 16. <sup>uh</sup> See ref 16. <sup>ui</sup> See ref 16. <sup>uj</sup> See ref 16. <sup>uk</sup> See ref 16. <sup>ul</sup> See ref 16. <sup>um</sup> See ref 16. <sup>un</sup> See ref 16. <sup>uo</sup> See ref 16. <sup>up</sup> See ref 16. <sup>uq</sup> See ref 16. <sup>ur</sup> See ref 16. <sup>us</sup> See ref 16. <sup>ut</sup> See ref 16. <sup>uu</sup> See ref 16. <sup>uv</sup> See ref 16. <sup>uw</sup> See ref 16. <sup>ux</sup> See ref 16. <sup>uy</sup> See ref 16. <sup>uz</sup> See ref 16. <sup>va</sup> See ref 16. <sup>vb</sup> See ref 16. <sup>vc</sup> See ref 16. <sup>vd</sup> See ref 16. <sup>ve</sup> See ref 16. <sup>vf</sup> See ref 16. <sup>vg</sup> See ref 16. <sup>vh</sup> See ref 16. <sup>vi</sup> See ref 16. <sup>vj</sup> See ref 16. <sup>vk</sup> See ref 16. <sup>vl</sup> See ref 16. <sup>vm</sup> See ref 16. <sup>vn</sup> See ref 16. <sup>vo</sup> See ref 16. <sup>vp</sup> See ref 16. <sup>vq</sup> See ref 16. <sup>vr</sup> See ref 16. <sup>vs</sup> See ref 16. <sup>vt</sup> See ref 16. <sup>vu</sup> See ref 16. <sup>vv</sup> See ref 16. <sup>vw</sup> See ref 16. <sup>vx</sup> See ref 16. <sup>vy</sup> See ref 16. <sup>vz</sup> See ref 16. <sup>wa</sup> See ref 16. <sup>wb</sup> See ref 16. <sup>wc</sup> See ref 16. <sup>wd</sup> See ref 16. <sup>we</sup> See ref 16. <sup>wf</sup> See ref 16. <sup>wg</sup> See ref 16. <sup>wh</sup> See ref 16. <sup>wi</sup> See ref 16. <sup>wj</sup> See ref 16. <sup>wk</sup> See ref 16. <sup>wl</sup> See ref 16. <sup>wm</sup> See ref 16. <sup>wn</sup> See ref 16. <sup>wo</sup> See ref 16. <sup>wp</sup> See ref 16. <sup>wq</sup> See ref 16. <sup>wr</sup> See ref 16. <sup>ws</sup> See ref 16. <sup>wt</sup> See ref 16. <sup>wu</sup> See ref 16. <sup>wv</sup> See ref 16. <sup>wx</sup> See ref 16. <sup>wy</sup> See ref 16. <sup>wz</sup> See ref 16. <sup>xa</sup> See ref 16. <sup>xb</sup> See ref 16. <sup>xc</sup> See ref 16. <sup>xd</sup> See ref 16. <sup>xe</sup> See ref 16. <sup>xf</sup> See ref 16. <sup>yg</sup> See ref 16. <sup>yh</sup> See ref 16. <sup>yi</sup> See ref 16. <sup>yj</sup> See ref 16. <sup>yk</sup> See ref 16. <sup>yl</sup> See ref 16. <sup>ym</sup> See ref 16. <sup>yn</sup> See ref 16. <sup>yo</sup> See ref 16. <sup>yp</sup> See ref 16. <sup>yq</sup> See ref 16. <sup>yr</sup> See ref 16. <sup>ys</sup> See ref 16. <sup>yt</sup> See ref 16. <sup>yu</sup> See ref 16. <sup>yv</sup> See ref 16. <sup>yw</sup> See ref 16. <sup>yx</sup> See ref 16. <sup>yy</sup> See ref 16. <sup>yz</sup> See ref 16. <sup>za</sup> See ref 16. <sup>zb</sup> See ref 16. <sup>zc</sup> See ref 16. <sup>zd</sup> See ref 16. <sup>ze</sup> See ref 16. <sup>zf</sup> See ref 16. <sup>zg</sup> See ref 16. <sup>zh</sup> See ref 16. <sup>zi</sup> See ref 16. <sup>zj</sup> See ref 16. <sup>zk</sup> See ref 16. <sup>zl</sup> See ref 16. <sup>zm</sup> See ref 16. <sup>zn</sup> See ref 16. <sup>zo</sup> See ref 16. <sup>zp</sup> See ref 16. <sup>zq</sup> See ref 16. <sup>zr</sup> See ref 16. <sup>zs</sup> See ref 16. <sup>zt</sup> See ref 16. <sup>zu</sup> See ref 16. <sup>zv</sup> See ref 16. <sup>zw</sup> See ref 16. <sup>zx</sup> See ref 16. <sup>zy</sup> See ref 16. <sup>zz</sup> See ref 16. <sup>aa</sup> See ref 16. <sup>ab</sup> See ref 16. <sup>ac</sup> See ref 16. <sup>ad</sup> See ref 16. <sup>ae</sup> See ref 16. <sup>af</sup> See ref 16. <sup>ag</sup> See ref 16. <sup>ah</sup> See ref 16.

Table II. Synthesis of 5-Substituted Deoxypyrimidine Nucleosides

product	starting materials <sup>a</sup>	method (equivalents of catalyst <sup>b</sup> )	reaction time, h	yield, %
7	3a + CH <sub>2</sub> =CHCN (7.0)	A (1.0)	12	16 <sup>c</sup>
8	3a + CH <sub>2</sub> =CHCO <sub>2</sub> CH <sub>3</sub> (5.0)	A (0.25) + CuCl <sub>2</sub> (1.0)	3	60 <sup>b</sup>
9	3a + CH <sub>2</sub> =CHCH <sub>2</sub> Cl (9.5)	A (0.5)	0.5	71 <sup>c</sup>
10	9	D (0.14)	16	29 <sup>b</sup>
12	1b + CH <sub>2</sub> =OCH <sub>3</sub> (excess)	C (0.25) + CuI (0.1)	7	60 <sup>c</sup>
14 <sup>d</sup>	3b + CH <sub>2</sub> =CHCH <sub>2</sub> Cl (8.4)	A (0.44) + CuCl <sub>2</sub> (1.1) and D (0.1)	3, 16	42 <sup>b</sup> 46 <sup>b</sup>
16	3a + CH <sub>2</sub> =C(CH <sub>3</sub> )CH <sub>2</sub> Cl (9.0)	A (1.0)	24	21 <sup>c</sup>
20	3a + CH <sub>2</sub> =CHCH <sub>2</sub> CO <sub>2</sub> CH <sub>3</sub> (9.0)	A (1.0) + CuCl <sub>2</sub> (1.0)	96	21 <sup>b</sup>
21	3a + CH <sub>2</sub> =C(CH <sub>3</sub> )CO <sub>2</sub> CH <sub>3</sub> (4.5)	A (1.0)	16	63 <sup>c</sup>
22	3a + CH <sub>2</sub> =CHCH <sub>2</sub> Cl (9.0)	A (0.25)	0.5	58 <sup>c</sup>
23	22	D (0.1)	16	12 <sup>b</sup>
25	1b + CH <sub>2</sub> =OCH <sub>3</sub> (excess)	C (0.25) + CuI (0.05)	9	28 <sup>c</sup>
26	3a + CH <sub>2</sub> =C(CH <sub>3</sub> )CH <sub>2</sub> Cl (9.5)	A (0.3) and D (0.15)	4, 16	30, <sup>a</sup> 61 <sup>c,e</sup>
27 <sup>f</sup>	3a + CH <sub>2</sub> =CHCH <sub>2</sub> Cl (8.7)	A (0.2) and D (0.1)	0.5, 16	15 <sup>a</sup> , 18 <sup>c,e</sup> 44 <sup>a,d</sup>
29	2a + CH <sub>2</sub> =CHCH(OTf)CH <sub>2</sub> CH <sub>3</sub> (5.3)	A (0.25) and D (0.1)	2.5, 18	80, <sup>d</sup> 36 <sup>c,e</sup>
29	1a + CH <sub>2</sub> =CHCH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub> (5.0)	B (0.1)	12	25 <sup>c</sup>
30	1a + CH <sub>2</sub> =CH(CH <sub>2</sub> ) <sub>3</sub> CH <sub>3</sub> (16)	B (0.1)	2	15 <sup>c</sup>
31	1a + CH <sub>2</sub> =CHC(CH <sub>3</sub> ) <sub>2</sub> CH <sub>3</sub> (12)	B (0.08)	48	16 <sup>c</sup>
32	2 + CH <sub>2</sub> =CHCO <sub>2</sub> CH <sub>3</sub> (3.7)	f (0.06)	2.5	55 <sup>c</sup>

<sup>a</sup> Figures in parentheses are equivalents of reagent and catalyst to 1 equiv of nucleoside. <sup>b</sup> Yield after recrystallization.<sup>c</sup> Yield after chromatography. <sup>d</sup> Yield for first stage of the preparation. <sup>e</sup> Yield for second stage of the preparation.<sup>f</sup> For method see text.

readily carried out with Wilkinson's catalyst.<sup>17</sup> Most of the compounds under study were prepared by such palladium-catalyzed reactions, which are summarized in Table II. 5-(4-Carbomethoxybut-1,3-dienyl)-2'-deoxyuridine (32) was synthesized by the analogous reaction of the vinyl bromide (2) and methyl acrylate. Those compounds with vinyl halide substituents, i.e., BVDU (2) and the methyl derivatives (17-19), were prepared from the corresponding acrylate esters by hydrolysis-halogenation procedures.<sup>18</sup> A Wittig reaction on 5-formyl-2'-deoxyuridine gave the (E)-5-[2-(methylthio)vinyl]-2'-deoxyuridine (6).

The stereochemical assignments of the compounds in Table I have been made, where appropriate, from <sup>1</sup>H NMR and UV data. The disubstituted alkenes 2, 6-8, 10, 14, 23, and 29-32 have coupling constants for the olefinic protons of *J* = 16-18 Hz and have been assigned the *E* stereochemistry. Several isomeric compounds, 4, 11, and 15, with *J* = 8-11 Hz are of the *Z* configuration. In order to ascertain the geometry of the trisubstituted alkenes, nuclear Overhauser enhancements (NOE) of the various side-chain protons were determined during irradiation of the base proton H-6. The occurrence of positive NOE effects on the vinyl protons of 17, 19, 20, and 27 and on the methyl group of 21 indicated that these were *E* isomers, while a positive NOE effect on the terminal methyl group of 28 indicated that this was the *Z* isomer.

Further structural information for the 5-(substituted-alkenyl)pyrimidine nucleosides has been gained from  $\lambda_{\max}$  values. A substituent in conjugation with the pyrimidine ring results in a bathochromic shift of >20 nm relative to either the parent nucleoside or its 5-alkyl derivatives [compare 5-(1-butenyl)-2'-deoxyuridine (23),  $\lambda_{\max}$  295 nm, with 5-(2-butenyl)-2'-deoxyuridine (22),  $\lambda_{\max}$  270 nm, and 5-butenyl-2'-deoxyuridine (24),  $\lambda_{\max}$  270 nm]. In addition, the  $\lambda_{\max}$  of *E* isomers are approximately 5 nm greater than those of the *Z* isomers (compare 2 and 4, 10 and 11, 14 and 15, and 27 and 28, Table I). These observations are viewed in terms of steric interactions in the *Z* isomers between

either the 2-bromo or 2-methyl groups and the pyrimidine ring atoms which prevent the side chain adopting a conformation coplanar with the ring. The consequent reduction in the extent of conjugation is reflected in the reduced  $\lambda_{\max}$  values.

## Results and Discussion

The compounds were tested for antiviral activity against HSV-1 in a micropaque reduction assay. The antiviral activities of 2, 4, 9, and 10 have already been reported.<sup>10,19-21</sup> and 2 is the most effective inhibitor of HSV-1 replication in cell culture so far described. The minimum inhibitory concentration (MIC) for each compound is shown in Table I. For comparison, 1a has an MIC of 0.5  $\mu$ g/mL against HSV-1 in the test system employed here. An analysis of the results of the antiviral evaluations indicated that certain features were required in the substituent of 5-modified deoxyuridines for optimum antih herpes activity, whereas other features were undesirable. Those features enhancing or reducing antiviral activity are as follows.

(1) **Conjugation.** There was a considerable increase in antiviral activity against HSV-1 when the double bond in the side chain of 9 or 22 was brought into conjugation with the pyrimidine ring, as in 10 and 23, respectively. The saturated butyl derivative 24 had similar anti-HSV-1 activity to the nonconjugated alkene 22. A similar effect is also seen with the saturated propyl (not shown here), which is less active than the conjugated alkene 10.<sup>6</sup> The propyne (12) showed no additional advantage over the propene (10) against HSV-1, with a minimal difference in MICs, but the butyne (25) was significantly less active than the butene (23).

(2) **Chain Length.** In a homologous series of side chains from propene to hexene, it was observed that extending the side chain longer than four carbons caused a

[17] Ruth, J. L.; Bergstrom, D. E. *J. Org. Chem.* 1978, 43, 2870-2878.[18] Jones, A. S.; Verhelst, G.; Walker, R. T. *Tetrahedron Lett.* 1979, 4415-4418.[19] Jones, A. S.; Rahim, S. G.; Walker, R. T.; De Clercq, E. *J. Med. Chem.* 1981, 24, 759-760.[20] Cerog, Y.-C.; Domin, B. A.; Sharma, R. A.; Bobek, M. *Antimicrob. Agents Chemother.* 1978, 10, 119-122.[21] Chang, Y.-C.; Grill, S.; Ruth, J.; Bergstrom, D. E. *Antimicrob. Agents Chemother.* 1980, 18, 957-961.

sharp cutoff in antiviral activity. In vitro, the propene (10) and butene (23) were equally effective against HSV-1, while the pentene (29) and hexene (30) were without antiviral activity at the maximum concentration tested. Extension of the side chain in 8 by another vinyl group to give 32 also resulted in a loss of antiviral activity.

(3) *E/Z* Isomerization. In all four cases where both *E* and *Z* isomers of a compound were tested, the *E* isomer was more active against HSV-1 than the *Z* isomer. This was true for both antiviral deoxyuridines (compare 2 with 4, 10 with 11, and 27 with 28) and deoxycytidines (compare 14 and 15) and was particularly marked in the case of 10 and 11.

(4) Substitution of Br in Compound 2. Replacement of the bromine in 2 with either nitrile (7) or methyl (10), both of which have a smaller molar refraction (an estimate of steric bulk) than bromine, failed to afford a more inhibitory compound. If, in this instance, size is not a constraint on antiviral activity, then other properties of bromine, not shared with nitrile or methyl, may also be important in conferring antiviral properties on the substituted nucleosides. In particular, the hydrophobicity and inductive effects of bromine may be important. The reduced antiviral activity of 7 compared with 2 may be due to the hydrophilic nature of nitriles, and it is worthy of note that the use of the less hydrophobic halogen chlorine gave a less active antiviral compound than 2.<sup>22</sup> The superior activity of 2 compared with 10 cannot be readily explained in terms of hydrophobic properties, methyl and bromine being very similar in this respect, but may be attributable to the inductive property of bromine not found with methyl. While the greater size of thiomethyl (6) and methoxycarbonyl (8) may be of prime importance in limiting their antiviral activity compared with 2, the superior activity of 6 compared to 8 may be because of the hydrophobic nature of the former.

(5) Branching. The antiviral activity of 16 was substantially reduced when either of the olefinic carbon atoms in the side chain were methylated (see 26 and 27) or when the terminal carbon was a branching point (31). Methylation of either olefinic carbon in 8 produced a similar effect (see 20 and 21). The introduction into 2 of vinyl methyl groups was not advantageous in that 17 was less active than 2 and, similarly, 19 was less active than the equivalent (*E*)-5-(2-chlorovinyl)-2'-deoxyuridine.<sup>22</sup> A mixture of *E* and *Z* isomers of the 2-methylated derivative 18 was less active than either isomer (2 and 4) of the parent compound. The introduction of a second bromine atom (5) resulted in a compound that was less active than the monohaloalkenylated *E* isomer 2 but more active than 18, whose bromo and methyl groups should have very similar steric requirements to the two bromine atoms. The antiviral activity of 5 might, therefore, reflect the opposing effects of the electronic properties (beneficial) of the second bromine atom and its steric requirements (deleterious).

(6) Replacement of O at C-4 in Compound 10. 5-Substituted deoxycytidine derivatives have not been investigated as antiherpetic compounds to the same extent as deoxyuridines. Although some have been found to be as inhibitory toward HSV-1 as their deoxyuridine equivalents, this is not always the case.<sup>23</sup> Here we found that 5-propenyl-2'-deoxycytidine (14) was less active than the

corresponding compound (10) in the deoxyuridine series. The 4-thiouracil derivative (13) was less active than either the uracil or cytosine analogues.

## Experimental Section

Melting points were determined on an Electrothermal apparatus and are not corrected. Ultraviolet spectra were recorded in methanol with a Pye-Unicam SP1800 spectrophotometer. All compounds were characterized by NMR on a Varian FT80A instrument and by chemical-ionization mass spectrometry on a Finnigan 4000 instrument and have elemental analysis correct to within  $\pm 0.4\%$  unless stated otherwise. Nuclear Overhauser experiments were carried out on a Bruker WM250 instrument. Compounds were prepared according to general methods (A-D) or specific methods described.

**Method A. (*E*)-5-(2-Carbomethoxy-2-methylvinyl)-2'-deoxyuridine (21).** Methyl methacrylate (1 mL, 9.35 mmol),  $\text{Sn}^{4+}$  (4 g, 2.15 mmol), and a 0.1 M methanolic solution of  $\text{LiPF}_6\text{Cl}_4$  (22 mL, 2.2 mmol) (together with copper(II) chloride where indicated in Table II) were stirred together at room temperature for 16 h. Hydrogen sulfide was passed through the solution for 1–2 min, followed by nitrogen (to remove excess  $\text{H}_2\text{S}$ ), and the precipitated metal sulfides were removed by filtration through Celite. The filtrate was basified with methanolic ammonia and filtered, and then the filtrate was concentrated under vacuum. The product was chromatographed on silica in  $\text{CHCl}_3/\text{EtOH}$  to give 21 (0.70 g, 83%), which crystallized from water, mp 181–182 °C. Anal. ( $\text{C}_{14}\text{H}_{20}\text{N}_2\text{O}_7$ )  $\text{C}, \text{H}, \text{N}$ .

**Method B. (*E*)-5-(3-Dimethyl-1-butenyl)-2'-deoxyuridine (31).** Compound 1a (500 mg, 1.41 mmol), palladium(II) acetate (25 mg, 0.11 mmol), triethylamine (0.7 mL, 5 mmol), 3,3-dimethyl-1-butene (2 mL, 18.83 mmol), and  $\text{CH}_3\text{CN}$  (5 mL) were heated at 100 °C in a steel pressure reactor for 48 h. The palladium residues were removed by centrifugation, and the supernatant was evaporated under vacuum and purified on a column of ODS reverse-phase silica by using a gradient of 10–20% methanol in water. This gave 70 mg (16%) of 31, which was crystallized from water to give an analytically pure sample, mp 180–182 °C. Anal. ( $\text{C}_{16}\text{H}_{24}\text{N}_2\text{O}_7$ )  $\text{C}, \text{H}, \text{N}$ .

**Method C. 5-(1-Propenyl)-2'-deoxyuridine (12).** Propylene gas was slowly bubbled through a vigorously stirred mixture of 3,5-di-*o*-benzoyl-6-iodo-2'-deoxyuridine (prepared from 1a and 2.0 equiv of benzoyl chloride by conventional means,<sup>22</sup> 500 mg, 1 mmol),  $\text{Pd}(\text{PPh}_3)_4$  (100 mg, 0.25 mmol), and  $\text{CuI}$  (60 mg, 0.10 mmol) in  $\text{Et}_3\text{N}$  (200 mL) at 25 °C for 7 h. The solvent was evaporated under vacuum, and the residue was refluxed with  $\text{NaOMe}$  (540 mg, 10 mmol) in  $\text{MeOH}$  for 30 min. The solution was neutralized with Dowex 50W-X8 ( $\text{H}^+$ ) ion-exchange resin, and the product was chromatographed on ODS reverse-phase silica (0–30% methanol in water gradient) to give 160 mg (80%) of 12. Crystallization from water gave an analytically pure sample, mp 181–182 °C. Anal. ( $\text{C}_{12}\text{H}_{16}\text{N}_2\text{O}_7$ )  $\text{C}, \text{H}, \text{N}$ .

**Method D. Preparation of (*E*)-5-(1-Propenyl)-2'-deoxyuridine (10) by Isomerization of Compound 9.** Compound 9<sup>17</sup> (1.3 g, 4.86 mmol) and  $\text{RuCl}_2(\text{PPh}_3)_3$  (prepared by the method of Bennett and Longstaff,<sup>24</sup> 278 mg, 0.6 mmol) were refluxed in  $\text{EtOH}$  (50 mL) for 16 h. The solvent was evaporated under vacuum, and the residue was crystallized from  $\text{CH}_2\text{Cl}_2$  to give 364 mg (29%) of 10; mp 191–192 °C. Anal. ( $\text{C}_{12}\text{H}_{16}\text{N}_2\text{O}_7$ )  $\text{C}, \text{H}, \text{N}$ .

**(*E*)-5-(2-Bromo-2-methylvinyl)-2'-deoxyuridine (17).** An aqueous solution of sodium hydroxide (0.1 M) was added dropwise to a solution of 26 (0.61 g, 1.87 mmol) in water (30 mL) until no starting material remained by TLC. The solution was neutralized with  $\text{AcOH}$  and then heated to 70 °C. *N*-Bromosuccinimide (0.33 g, 1.85 mmol) was added in portions over 30 min to the hot stirred solution. The reaction mixture was cooled and evaporated to dryness, and the product was chromatographed on silica in

(22) Walker, R. T.; Jones, A. S.; De Clercq, E.; Descamps, J.; Al-lauden, H. S.; Koczarik, W. W. *Nucleic Acids Symp. Ser.* 1980, no. 8, 895–9102.

(23) De Clercq, E.; Balzarini, J.; Descamps, J.; Huang, G.-F.; Torrence, P. F.; Bergstrom, D. E.; Jones, A. S.; Sarrafian, P.; Verheist, G.; Walker, R. T. *Mol. Pharmacol.* 1982, 21, 217–223.

(24) Bergstrom, D. E.; Rith, J. L. *J. Carbohydr. Nucleosides, Nucleotides* 1977, 4, 287–289.

(25) Garrett, P. E. *Synth. Proc. Nucleic Acid Chem.* 1968, 1, 459–440.

(26) Bennett, M. A.; Longstaff, P. A. *Chem. Ind. (London)* 1968, 846.

$\text{CHCl}_3/\text{EtOH}$  (8:1). Recrystallization from water gave 91 mg (15%) of 17 as pale yellow plates, mp 130–131 °C dec. Anal. ( $\text{C}_{19}\text{H}_{20}\text{BrN}_2\text{O}_3$ ) C, H, N, Br.

Compound 18 was prepared by the above procedure from compound 21 (500 mg, 1.5 mmol): yield of 18 after recrystallization from water 85 mg (18%); mp 194–196 °C. Anal. ( $\text{C}_{12}\text{H}_{14}\text{BrN}_2\text{O}_3$ ) C, H, N, Br.

Compound 19 was prepared by a similar method from 20 (3.0 g, 5.1 mmol) and *N*-chlorosuccinimide (1.06 g, 5 mmol): yield of 19 after recrystallization from water 307 mg (16%); mp 148–149 °C. Anal. ( $\text{C}_{19}\text{H}_{21}\text{ClN}_2\text{O}_3$ ) C, H, N, Cl.

5-(2-Dibromovinyl)-2'-deoxyuridine (5). A solution of bromine in DMF was added dropwise to a solution of 2 (166 mg, 0.48 mmol) in DMF (2 mL) at 5 °C until TLC showed that no starting material remained. The solution was then heated at 100 °C for 25 min, the solvent was removed under vacuum, and the product was purified on silica in  $\text{CHCl}_3/\text{EtOH}$  to give 5, yield 83 mg (42%). Recrystallization from  $\text{CH}_2\text{Cl}_2/\text{EtOH}$  gave an analytically pure sample, mp 184.5–185 °C. Anal. ( $\text{C}_{12}\text{H}_{12}\text{Br}_2\text{N}_2\text{O}_3$ ) C, H, N, Br.

(E)-5-[2-(Methylthio)vinyl]-2'-deoxyuridine (6). A solution of [(methylthio)methyl]triphenylphosphonium chloride<sup>27</sup> (1.23 g, 3.44 mmol) in dry MeOH (4 mL) under nitrogen was treated with a freshly prepared 0.8 M methanolic solution of NaOMe (4.7 mL, 3.44 mmol) at 20 °C to give a white precipitate. To this was added a solution of 3',5'-di-O-acetyl-6-formyl-2'-deoxyuridine<sup>28</sup> (68 mg, 1.72 mmol) in MeOH (5 mL), and the reaction was stirred for 15 h. After neutralization with Dowex 50W-X8 ( $\text{H}^+$ ) ion-exchange resin, the solution was concentrated under vacuum when the product separated as crystals. Recrystallization from MeOH gave 6: yield 311 mg (80%); mp 219–220 °C. Anal. ( $\text{C}_{13}\text{H}_{16}\text{N}_2\text{O}_5\text{S}$ ) C, H, N, S.

(Z)-5-Propenyl-2'-deoxyuridine (11). The E isomer (10; 1 g, 3.73 mmol) and benzophenone (1 g) in MeOH (100 mL) were irradiated with a 6-W mercury UV lamp (quartz filter) for 24 h. The solvent was evaporated under vacuum, and the residue was chromatographed on ODS reverse-phase silica using an aqueous methanol (10–30% methanol) gradient. The Z isomer, which eluted before the starting material, was recrystallized from aqueous methanol: yield 110 mg (11%); mp 171–173.5 °C. Anal. ( $\text{C}_{12}\text{H}_{16}\text{N}_2\text{O}_3$ ) C, H, N.

Preparation of (E)-5-(1-Propenyl)-4-thio-2'-deoxyuridine (13). 3',5'-Di-O-benzoyl-5-(1-propenyl)-2'-deoxyuridine (prepared from 10 and 2.0 equiv of benzoyl chloride by conventional means;<sup>28</sup> 5.0 g, 12.6 mmol) was refluxed with phosphorus pentasulfide (20 g) in pyridine (350 mL) with vigorous mechanical stirring for 4 h. The solvent was evaporated under vacuum, and the residual oil was poured into water (1 L) and then extracted with  $\text{CHCl}_3$  (3 × 100 mL). 4-Thio-3',5'-di-O-benzoyl-5-(1-propenyl)-2'-deoxyuridine was obtained as a pale yellow solid from the organic extract and was recrystallized from EtOH to give 3.9 g (63%), mp 88–90 °C. The protected nucleoside (500 mg, 1 mmol) was stirred in 1 M methanolic NaOMe (5 mL) at room temperature for 12 h. The solution was neutralized by passing through a column of Dowex 50W-X8 ( $\text{H}^+$ ) ion-exchange resin, and solvent was removed under vacuum. The product was chromatographed on silica in  $\text{CHCl}_3/\text{EtOH}$  to give 180 mg (64%) of 13. Recrystallization from  $\text{CH}_2\text{Cl}_2/\text{EtOH}$  gave an analytically pure sample, mp 117–118 °C. Anal. ( $\text{C}_{17}\text{H}_{18}\text{N}_2\text{O}_5\text{S}$ ) C, H, N, S.

5-Butyl-2'-deoxyuridine (24). A methanolic solution of 22 (1.52 g, 5.4 mmol) was stirred with 10% Pd on charcoal (200 mg)

in an atmosphere of hydrogen until 1 equiv of gas was absorbed (10 min). The catalyst was removed by filtration through Celite, and the filtrate was evaporated to dryness. The residue was recrystallized from  $\text{CH}_2\text{Cl}_2$  to give 24, 1.02 g (57%), mp 121 °C. Anal. ( $\text{C}_{19}\text{H}_{24}\text{N}_2\text{O}_3$ ) H, N, C: calcd, 54.92; found, 54.33.

3-(Toluenesulfonyloxy)pent-1-ene. 1-Pent-3-ol (8.6 g, 0.1 mmol) was treated with tosyl chloride (22.3 g, 0.12 mmol) in pyridine (200 mL) at 20 °C for 16 h. The reaction products were poured into water, and the tosylate was extracted with ether. The organic phase was washed with dilute HCl and water and then dried. Evaporation of the solvent under vacuum below room temperature gave the tosylate, 4.8 g (20%), as a mobile yellow liquid: NMR ( $\text{CDCl}_3$ )  $\delta$  1.0 (3 H, t,  $J = 7$  Hz,  $\text{CH}_3$ ), 1.85 (2 H, m,  $\text{CH}_2$ ), 2.47 (3 H, s,  $\text{CH}_3\text{-Ar}$ ), 4.20 (1 H, m,  $\text{CHOTf}$ ), 5.5 (3 H, m,  $\text{CH}_2\text{-Ar}$ ), 5.5 (1 H, m,  $-\text{CH}$ ), 7.35 (3 H, d,  $J = 10$  Hz,  $\text{Ar}$ ), 7.37 (2 H, d,  $J = 10$  Hz,  $\text{Ar}$ ). The tosylate was not purified further but used directly for the preparation of 29 by method A. Yields from this latter reaction were variable, possibly as a result of instability of the tosylate.

(E,E)-5-(4-Carbomethoxy-1,3-butadienyl)-2'-deoxyuridine (32). Palladium(II) acetate (180 mg, 0.7 mmol), triphenylphosphine (380 mg, 1.45 mmol), and triethylamine (3.5 mL) were heated in refluxing dioxane (15 mL) for 20 min to give a black solution. A suspension of 2 (5 g, 18 mmol) in dioxane (20 mL) was added, followed by methyl acetate (5 mL, 55.5 mmol), and the reaction mixture was vigorously stirred and heated under reflux for a further 6 h. The palladium residues were filtered from the hot reaction mixture, and the filtrate was evaporated to dryness under vacuum and washed with  $\text{CH}_2\text{Cl}_2$  (2 × 50 mL). Chromatography on silica in  $\text{CH}_2\text{Cl}_2/\text{EtOH}$  gave 3.2 g (55%) of 32, which was recrystallized from MeOH to give pale yellow needles, mp 188 °C dec. Anal. ( $\text{C}_{23}\text{H}_{28}\text{N}_2\text{O}_7$ ) C, H, N.

Antiviral Assays. The antiviral activity of the compounds was assessed by a microplate reduction method using the S9 strain of HSV-1.<sup>29</sup> Virus infectivity end-point titrations were performed by inoculation of serial 0.5 log dilutions of virus on monolayers of baby hamster kidney cells<sup>30</sup> in flat-bottomed microtiter plates (Flow Laboratories, Irvine, Scotland). Test compounds were included at the required concentration in the overlay (Eagles minimal essential medium, Dulbecco's modification, containing 10% donor calf serum (Flow Laboratories) (growth medium) and 0.5% carboxymethylcellulose (Sigma Chemical Co.). The minimum inhibitory concentration (MIC) of each compound was determined to be the least concentration that gave a reduction in virus infectivity end point of not less than 1 log compared with the non-drug-treated control.

Registry No. 1a, 54-42-5; 1b, 4833-07-2; 2, 69304-47-8; 8a, 85506-76-2; 3b, 85523-09-3; 4, 77330-02-0; 5, 61135-34-2; 6, 86163-16-8; 7, 80179-35-9; 8, 86163-17-9; 9, 75-39-2; 10, 69270-29-9; 11, 69872-52-1; 12, 84558-94-1; 13, 85176-91-2; 14, 69270-34-6; 15, 69270-35-7; 16, 86163-18-0; 17, 86163-19-1; (E)-18, 86163-20-4; (Z)-18, 86163-34-0; 19, 86163-21-5; 20, 86163-22-8; 21, 86163-23-7; (E)-22, 76334-43-5; (Z)-22, 76334-44-3; 23, 85328-78-5; 24, 57741-91-0; 25, 77875-96-8; 26, 86163-24-8; 27, 86163-25-9; 28, 86163-26-0; 29, 86163-27-1; 30, 81710-43-2; 31, 86163-28-2; 32, 86163-29-3; methyl methacrylate, 96-02-6; 3,3-dimethyl-2-butenes, 558-37-2; propyne, 74-90-4; 3',5'-di-O-benzoyl-5-formyl-2'-deoxyuridine, 86163-30-6; (E)-2',3'-di-O-benzoyl-5-(1-propenyl)-2'-deoxyuridine, 86163-31-7; (E)-4-thio-3',5'-di-O-benzoyl-5-(1-propenyl)-2'-deoxyuridine, 86163-32-8; 1-penten-3-ol, 616-26-1; 3-(tosyloxy)pent-1-ene, 86163-33-9.

(27) Wittig, G.; Schlosser, M. *Chem. Ber.* 1961, 94, 1379–1383.  
(28) Barwolf, D.; Laugen, P. *Nucleic Acid Chem.* 1978, 1, 359–366.

(29) Markham, A. F.; Newton, C. R.; Porter, R. A.; Sim, I. S. *Antiviral Res.* 1982, 2, 319–330.